Plant Cell and Tissue Culture

Dr. Omar M. Sabry

Plant Cell and Tissue Culture

What is the meaning of Plant cell and tissue culture?

In-vitro aseptic cultivation of isolated plant organs or cells (root tips, shoot tips, leaves, embryos, cells on a nutrient medium).

How can you overcome the problem of the difficulty of Taxol production, *in-vitro*?

Difficulty of Taxol production?

- We need 3 mature Taxus trees to get 1 gm taxol from their barks
- Each tree must be 100 years old for the bark to contain optimum amount of taxol
- A patient needs 2 gm taxol for full course treatment

Overcoming the problem

- We can produce taxol *in-vitro* by using plant cell culture technique to produce taxol in the cell culture laboratory

How can you Develop a commercial production of expensive and rare bio-medicaments by cell culture?

Taxol can be produced in few weeks by cell suspension culture. Previously, taxol was obtained from bark of 100-year-old *Taxus brevifolia* tree.

How can you make a medicinal plant like *Atropa belladonna* gives atropine and morphine together?

By producing transgenic *Atropa belladonna* through the transfer of the biosynthetic gene cluster of morphine from *Papaver sominfrum* by *Agrobacterium tumefaciens* to *Atropa belladonna* plant cells or by the molecular gene gun.

What are the Main units of Plant cell and tissue culture laboratory?

1- Washing area: To wash glassware and sterilize glassware by autoclave

2- Media room: To prepare media needed for culturing

3- Transfer room: To transfer explants to culture media under sterile conditions.

4- Culture room: Tissue culture and cell culture media are set for growth before transplantation

What is the Basic requirement of transfer area in plant cell and tissue culture?

Sterilization using laminar air flow cabinets and HEPA Filters

Why temperature and light source must be adjusted in culture room?

Temperature must be 25 - 26 ^{0}C and optimum growth Light must be present for photosynthesis

Why Borosilicate or Pyrex not Soda glassware should be used in tissue culture?

Soda glass is toxic to calli and cells as it is alkaline {releases sodium ion and hydroxide ions}, Pyrex and borosilicate are neutral.

What are the sources of tissue explants in plant cell and tissue culture technique?

Pith, root tips, shoot tips, embryos, buds, anthers and young leaves {Cut into 0.5 mm diameter cylindrical or cuboids pieces to allow penetration of nutrients, oxygen and water}.

Why tissues must be surface sterilized before planting on the nutrient medium?

To prevent microbial growth and spoilage of tissue cultures and plant cell cultures.

Why 70% ethyl alcohol then 1% Sodium hypochlorite is more preferable than 20% sodium hypochlorite in sterilization of explants?

20% Sodium hypochlorite is toxic causing discoloration and death of plant tissues.

Why Methanol can't be used for sterilization of explants?

Methanol is toxic producing formaldehyde causing damage of DNA {Death of tissues}.

Why After disinfection of explants, careful trimming must be carried out?

To remove the dead tissues specially after using Sodium hypochlorite.

Why we are Sterilizing glass ware, plastic ware and other instruments in plant cell and tissue culture technique?

To prevent contamination and microbial growth (Spoilage of tissue and plant cell cultures).

Why we are Sterilizing media used in plant cell and tissue culture technique?

To prevent contamination and microbial and fungal growth on the nutrient media (Spoilage of tissue and plant cell cultures).

What are the Media constituents in plant cell and tissue culture technique?

a- Inorganic Nutrients

Macro - or major elements: e.g. Nitrogen – Calcium used in formation of primary metabolites like proteins.

Micro - or minor elements: e.g. Iron - Cu used as co-enzymes {Redox enzymes}

b- Organic Nutrients

- Nitrogenous substances e.g. Vitamins {Thiamine - Pyrodoxol} and Hormones
- Carbon Source e.g. Sucrose 2-5%, glucose and fructose.

What are the Plant growth hormones in plant cell and tissue culture technique?

a- Cytokinins

Promotes growth of Shoots - e.g. BAP (Benzyl Amino Purine)

b- Auxins

Promotes growth of Roots - e.g. IAA (Indole Acetic Acid).

Why agar must be used in a concentration not more than 0.8 – 1.0 % in plant cell and tissue culture technique?

Upon using higher concentration, the medium becomes hard and will not allow the diffusion of nutrients into the tissues {Will not allow root growth}.

What are the factors inducing aging in plant cell and tissue culture technique?

a- Exhaustion of the nutrients

b- Inhibition of nutrient diffusion

c- Accumulation of toxic metabolites

d- Exhaustion of the Oxygen

Why activated charcoal may be added to cell suspension culture?

To remove accumulated toxins from the cell culture media and to prevent death of cells.

Why Callus tissues periodically transferred to fresh medium (sub-culture) at intervals of 4-6 weeks?

To avoid aging in plant cell and tissue culture which results from: Exhaustion of the nutrients - accumulation of toxic metabolites - exhaustion of the oxygen.

Why Agitation is important in cell suspension culture technique {Shakers}?

- To break clumps into single cells
- Achieve uniform distribution of cells in the medium
- Help in gaseous exchange process

What is meant by batch cell Culture?

A batch culture initiated by the transfer of a small portion of a culture into a new culture medium, resulting in growth and an increase in biomass. Biomass concentration can be measured in many ways, as cell number, dry weight, or in terms of any convenient biochemical component or parameter.

Advantages: Reduced risk of contamination

Disadvantages: Lower productivity levels

What is meant by continuous cell culture?

Rely on some type of cell retention mechanism that permits protein products to pass through to a collection system outside the bioreactor, while keeping productive cells inside. This process, known as perfusion cell culture, forms the basis of most continuous cultures.

Advantages: Results more consistent and higher productivity

Disadvantages: The original product strain could be lost over time

Why some cell cultures may not produce the natural compounds or produce them in very small amounts?

Compound may be produced in only one organ, or in one cell in certain organ. Compound may be biosynthesized by cooperation of different organs e.g. *Catharanthus roseus* plant needs to be fully differentiated into root, stem and leaves for production of vincristine. So, it can't produce the alkaloid in the cell culture form.

How can convert Digitoxin to Digoxin by enzyme located in the cell culture of *Digitalis lanata*?

Digitoxin is less active and more toxic than Digoxin. Biotransformation of Digitoxin to digoxin by enzyme located in the cell culture of *Digitalis lanata* is carried out {Hydroxylation process in position 12 of the steroidal nucleus of Digitoxin converting it to Digoxin to increase the activity and reduce toxicity.

Why Cellulase and/or pectinase enzymes are used for preparation of protoplasts?

As both or each one is able to carry out lysis of the plant cell (Cellulase lyses cellulose cell wall and pectinase lyses the pectin cell wall of the plant cell) giving the protoplast.

What is meant by Protoplast fusion?

Plant protoplasts fuse with each other irrespective of their origin (Protoplast Fusion) spontaneously or by a stimulant {Electroporation = High voltage 3000-4000 volts for very short time} or by chemical methods {By changing pH and calcium concentration}.

What is meant by spontaneous and induced Protoplast fusion?

Spontaneous Protoplast fusion: Happens when leaving two protoplasts in a solution without any stimulation {very slow with low % fusion}.

Induced Protoplast fusion: Happens by certain chemicals (immersion in ethylene glycol) or Electroporation (application of high voltage). Induced Protoplast fusion is more favorable as the result of fusion is higher than spontaneous Protoplast fusion.

How can you Increase the rate of production of bioactive secondary metabolites in cell suspension culture?

Addition of high conc. of precursors (Mevalonic acid to increase the rate of production of steroids)
Addition of colchicine to valerian cell suspension cultures. Colchicine causes multiplication of the number of chromosomes in valerian cells {cell culture} many times increasing the rate of production of valepotriates 60 times by increasing the number of the biosynthetic genes in the culture media.

Why mevalonic acid is added to cell suspension culture of Digitalis?

Mevalonic acid is the precursor of steroidal compounds (e.g. cardiac glycosides). So addition of mevalonic acid to the culture media increases the rate of steroidal compounds production like cardiac glycosides.

How can you Produce a haploid plant?

This can be achieved by using pollen grains as explants for tissue culture (contain 1n chromosomes). This will give a Dwarf plant.

Why we are Adding tryptophan to the culture media of *Cinchona Ledegriana*?

Tryptophan is the precursor of cinchona alkaloids {e.g. Quinine}. Addition of tryptophan to the culture media of *Cinchona Ledegriana* increases the rate of production of Quinoline type cinchona alkaloids {e.g. Quinine}.

Why Addition of colchicine to cell suspension culture of *Valeriana spp.* increases the rate of production of valpotriates by 60 times?

Colchicine causes multiplication of the number of chromosomes in cell culture of valerian cells many times (Polyploidy), increasing the rate of production of valpotriates 60 times by increasing the number of the biosynthetic genes in the culture media.